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Abstract: Molecular-based detection of bacterial pathogens directly from clinical specimens permits rapid initiation of effective antimicrobial treatment and adequate patient management. Broad-range polymerase chain reaction (PCR) amplification of the 16S rRNA gene (16S rDNA qPCR) is used in many diagnostic laboratories as a complement to cultural identification of bacterial pathogens. However, efforts for automation of 16S rDNA PCR workflows are needed in order to reduce turnaround times and to enhance reproducibility and standardization of the technique. In this retrospective method evaluation study, clinical specimens (N = 499) from patients with suspected bacterial infections were used to evaluate 2 diagnostic semiautomated workflows for rapid bacterial pathogen detection. The workflows included automated DNA extraction (QIASymphony), 16S rDNA qPCR, fragment or melting curve analysis, and amplicon sequencing. Our results support the use of the 16S rDNA qPCR and fragment analysis workflow as it enabled rapid and accurate identification of bacterial pathogens in clinical specimens.

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High-throughput screening of bacterial pathogens in clinical specimens using 16S rDNA qPCR and fragment analysis

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ABSTRACT

Molecular-based detection of bacterial pathogens directly from clinical specimens permits rapid initiation of effective antimicrobial treatment and adequate patient management. Broad-range polymerase chain reaction (PCR) amplification of the 16S rRNA gene (16S rDNA qPCR) is used in many diagnostic laboratories as a complement to cultural identification of bacterial pathogens. However, efforts for automation of 16S rDNA PCR workflows are needed in order to reduce turnaround times and to enhance reproducibility and standardization of the technique. In this retrospective method evaluation study, clinical specimens ($N = 499$) from patients with suspected bacterial infections were used to evaluate 2 diagnostic semiautomated workflows for rapid bacterial pathogen detection. The workflows included automated DNA extraction (QIASymphony), 16S rDNA qPCR, fragment or melting curve analysis, and amplicon sequencing. Our results support the use of the 16S rDNA qPCR and fragment analysis workflow as it enabled rapid and accurate identification of bacterial pathogens in clinical specimens.

1. Introduction

Rapid diagnosis of bacterial infections is a key factor for patient management, and delays in initiating effective antimicrobial treatment result in increased patient morbidity and mortality (Iregui et al., 2002). Bacterial culture takes at least 24 to 48 h for bacterial identification and may be false negative when fastidious pathogens like *Kingella kingae* or *Mycoplasma* spp., noncultivable bacteria such as *Coxiella burnetiid*, or nonviable bacteria are present in a clinical specimen (Cherkaoui et al., 2009). In contrast, quantitative polymerase chain reaction (qPCR) detects bacterial DNA regardless of viability, which is particularly important when empiric antibiotic therapy has already been administered to patients (Wagner et al., 2018). Recently, many syndromic point-of-care tests were launched that enable the detection of a panel of viral, bacterial, and fungal pathogens (Buss et al., 2015; Leber et al., 2016; Popowitch et al., 2013). However, syndromic PCR panels or qPCR use primers and probes that target specific bacterial species, thereby missing unexpected or rare bacterial pathogens. In contrast, nonspecific, broad-range PCR amplification of the bacterial 16S rRNA gene (16S rDNA qPCR) enables the detection of all bacteria present in a clinical sample (Bosshard et al., 2004; Rampini et al., 2011; Sontakke et al., 2009; Srinivasan et al., 2015), though supposedly with less sensitivity than targeted qPCR (Morel et al., 2015). However, the universality of 16S rDNA qPCR renders it vulnerable to contamination. All bacterial DNA present in a clinical sample is amplified, including the DNA that is present in extraction chemicals and PCR reagents. This emphasizes the need for the use of proper controls (i.e., process and negative controls that allow monitoring the DNA background in the chemicals and reagents) when using 16S rDNA qPCR workflows for pathogen detection.

16S rDNA amplification in combination with Sanger sequencing has been established as the standard approach for culture-independent detection of bacterial pathogens in many clinical microbiology laboratories. The main advantages of Sanger sequencing compared with next-generation sequencing (NGS) platforms are read lengths of up to 1000 bp combined with high accuracy. Moreover, 16S rDNA amplicons can be rapidly sequenced (8 amplicons per hour) with low per-sample cost (Lam et al., 2012). However, Sanger sequencing is problematic when clinical specimens contain multiple bacterial species, such as stool or respiratory samples or in polymicrobial infections. In such cases, results obtained by Sanger sequencing are not interpretable and make it hard or even impossible to identify specific pathogens. Therefore, Sanger sequencing is primarily recommended for analysis of clinical specimens from normally sterile body sites. NGS, in contrast, can be used to identify multiple bacterial species in a clinical specimen. Moreover, laboratories with large sample numbers can use high-throughput NGS platforms (e.g., from Illumina or Thermo Fischer) that have

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comparatively high sequencing depth despite having short read lengths (Besser et al., 2017).

In the past years, manual DNA extraction from clinical specimens and 16S rDNA PCR was combined with polyacrylamide gel electrophoresis (i.e., CleanGel with silver staining) and Sanger sequencing for bacterial identification at the Institute of Medical Microbiology, University of Zurich (Rampini et al., 2011). This workflow is time consuming with a lot of "hands-on-time" from laboratory personnel as DNA extraction, pipetting of the PCR amplification mixture and CleanGel analysis are performed manually. As a consequence, results are often not delivered as rapidly as required for proper patient management and correct antibiotic treatment initiation.

With advances in high-throughput technologies, these steps can be incorporated into a robotic liquid handling system, enabling semiautomation of 16S rDNA sequencing. Automation of some steps in these workflows like DNA extraction and pipetting of the PCR setup speeds up workflows while enhancing reproducibility and standardization. Recently, dual priming oligonucleotides (DPO) for sensitive and specific 16S rDNA qPCR have been developed (Kommedal et al., 2012). DPO primers typically consist of a longer 5′ segment that enables stable annealing of the primer and a shorter 3′ segment that ensures target specific extension. The 2 functional segments are connected by 5 consecutive deoxyinosine bases, called poly (I) linker (Chun et al., 2007).

The detection of 16S rDNA amplification can be done by using a labeled probe or an intercalating fluorescent dye. However, as no universal 16S rDNA qPCR probe is available at present, an alternative represents the use of SYBR Green® as fluorophore in qPCR amplification. This approach is sensitive, though a disadvantage is that SYBR Green® binds nonspecifically to all double-stranded DNA molecules present in a clinical sample (including human DNA, DNA from extraction chemicals, and PCR reagents), thereby obscuring quantification cycle values in qPCR. Fragment analyzer capillary electrophoresis or melting curve analysis can be used to distinguish nonspecific amplification products from 16S rDNA qPCR products.

In this retrospective method evaluation study, we used clinical specimens from sterile body sites ($N = 499$) that had been previously analyzed by culture and a 16S rDNA PCR workflow. This established workflow was compared to 16S rDNA qPCR using DPO primers combined with SYBR Green® detection, fragment or melting curve analysis, and Sanger sequencing (Fig. 1).

2. Materials and methods

2.1. Study design, clinical specimens, and medical record review

The retrospective study included 499 clinical specimens from unique patients analyzed between 2013 and 2017 at the diagnostic laboratory of the Institute of Medical Microbiology (IMM), University of Zurich. Clinical samples were sent to the IMM from secondary and tertiary hospitals in the Zurich metropolitan area (Switzerland, Europe) for

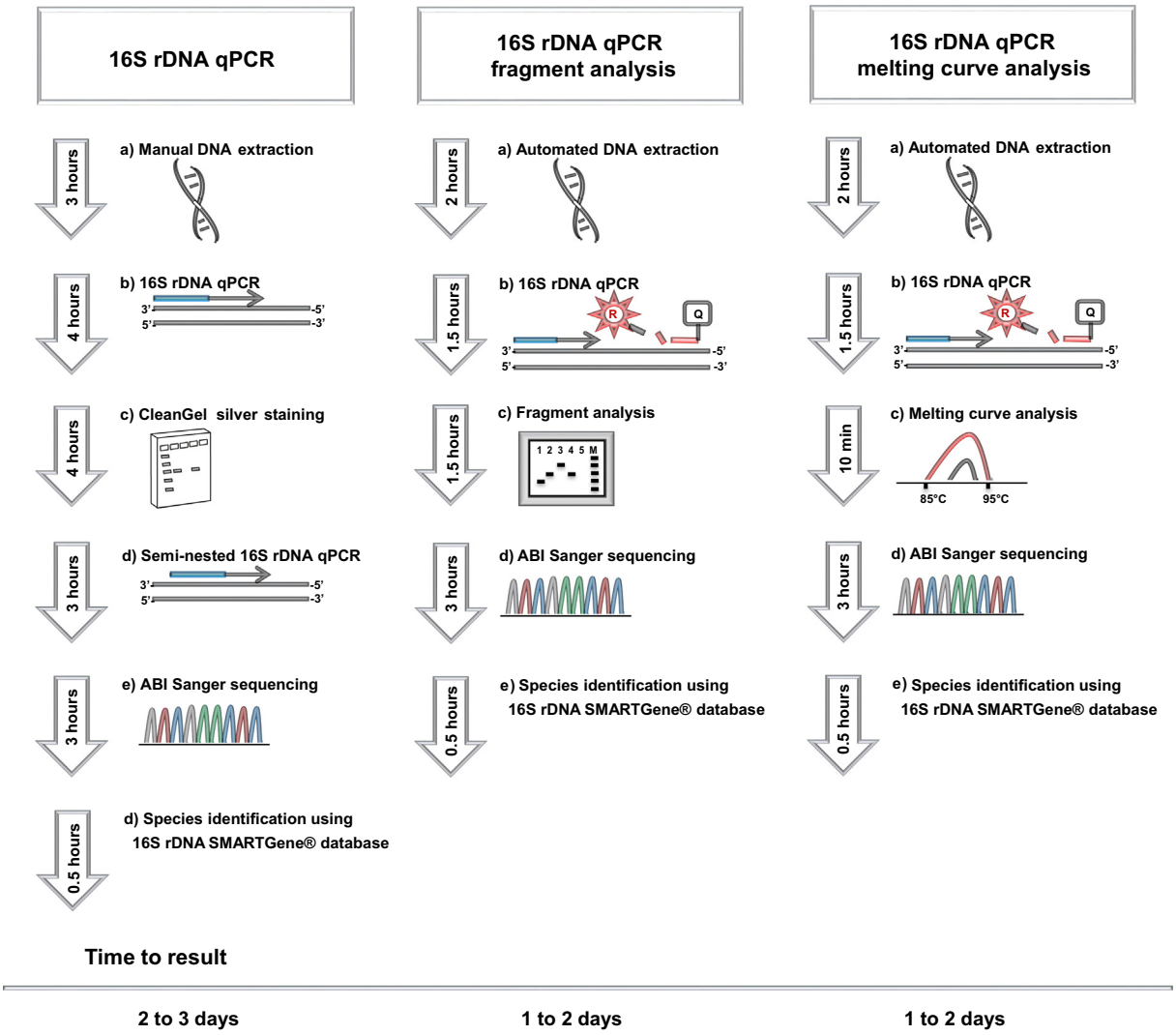

Fig. 1. Overview of the diagnostic scheme evaluated in this study for bacterial pathogen detection directly from clinical specimens (R refers to reporter; Q refers to quencher).

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bacterial identification by culture and amplification of the 16S rRNA gene (16S rDNA PCR). The specimens were solely from normally sterile body sites. We included the following sample types: fresh biopsies ($N = 215$), punctates and deep wound secretions ($N = 213$), whole blood ($N = 45$), bone marrow ($N = 2$), cerebrospinal fluid ($N = 17$), and bone samples ($N = 7$). Diagnosis of bacterial infection was established combining underlying disease and disease history of the patient, clinical course of disease and interventions, clinical signs and symptoms of inflammation, additional diagnostics if available (such as radiology and pathology reports), and microbiological findings (microscopy, culture, PCR results, detection of fungal or viral pathogens) including detailed consultations with the treating physicians. Therefore, all included specimens in the retrospective study were obtained from patients with a "confirmed bacterial infection" or "no bacterial infection".

2.2. Culture, microscopy, and 16S rDNA PCR workflow

Cultures were performed as described previously (Bosshard et al., 2003, 2004). In brief, aerobic culture was performed using Columbia blood agar, MacConkey agar, Columbia CNA blood agar, and Crowe agar (Becton Dickinson, Allschwil, Switzerland). Anaerobic culture was done using Brucella agar, kanamycin–vancomycin agar, and phenylethyl alcohol agar (Becton Dickinson, Allschwil, Switzerland), and thioglycolate broth was used for enrichment cultures. Agar plates were examined for growth after 24, 48, and 72 h. Cultures were assessed as negative if no bacterial growth was visible after 10 days of incubation in the liquid enrichment medium. Liquid samples were inoculated into aerobic and anaerobic BacT/Alert Blood Culture flasks (BioMérieux, Marcy-l'Etoile, France) for enrichment cultures and incubated in the BacT/Alert system for 6 days. Gram stains of clinical specimens were prepared according to standard procedures.

DNA extraction from clinical specimens and 16S rDNA PCR amplification were performed as described previously (Bosshard et al., 2003). *Escherichia coli* chromosomal DNA was used as positive control, and a water sample was used as negative control in each 16S rDNA PCR run. 16S rDNA PCR amplification products were visualized by polyacrylamide gel electrophoresis (CleanGel 10% 52S, ETC GmbH, Kirchentellinsfurt, Germany) combined with silver staining. Subsequently, a seminested PCR was performed; 16S rDNA PCR products were purified with the QIAquick PCR purification kit (Qiagen, Hombrechtikon, Switzerland) and sequenced with forward primer BAK11w (5′-AGTTTGATC[A/C]TGG CTCAG) using the BigDye kit (Life Technologies, Zug, Switzerland) and an automated DNA sequencer (ABI Prism 3130 Genetic Analyzer; Life Technologies, Zug, Switzerland). Bacterial identification was done using the 16S rDNA SmartGene IDNS custom platform following the identification guidelines published previously by Bosshard et al. (2003).

2.3. qPCR amplification of the 16S rRNA gene

DNA from clinical specimens was retrospectively analyzed by two 16S rDNA qPCR workflows (Fig. 1). For 16S rDNA qPCR, 5 μL of extracted DNA was added to a mixture consisting of 8.5 μL Roche water (Roche, Rotkreuz, Switzerland), 4 μL of a LightCycler® DNA multiplex master mix (Roche), 0.5 μM of each DPO® primer (DPO®-F: 5′-AGAGTTTGATCMTGGCTCA-I-I-I-I-I-AACGCT-3′, DPO®-R: 5′-CGCGGCTGCTGGCA-I-I-I-A-I-TTRGC-3′) (Kommedal et al., 2012), and 1 μM of SYBR Green (Sigma Aldrich, Buchs, Switzerland). qPCR amplification was done with a Lightcycler 480-II instrument (Roche). Cycling parameters included an initial denaturation for 5 min at 95 °C, followed by 45 cycles of 5 s at 95 °C, 15 s at 60 °C, and 15 s at 72 °C. The qPCR yielded a 500-bp PCR amplicon covering the V1 to V3 region of the 16S rRNA gene. All clinical specimens showed an amplification curve in qPCR analysis as even minimal traces of DNA were amplified and 16S rDNA fragments were subsequently analyzed by fragment or melting curve analysis. In each Lightcycler 480-II run, *E. coli* chromosomal DNA was included as positive control, and a water sample was included as negative control.

2.4. Fragment and melting curve analysis

Fragment analysis was done on a fragment analyzer automated CE system (Advanced Analytical Technologies Inc., Heidelberg, Germany) according to the manufacturer's instructions using the Fragment Analyzer-ds DNA 905 Kit. Clinical specimens were assessed as positive if they showed a fragment length of 450 bp to 500 bp and revealed greater fluorescence intensity (FI) than the water negative control.

Melting curve analysis was done on a Lightcycler 480-II instrument starting from 40 °C and increasing the temperature by 1.5 °C per second until a final temperature of 97 °C was reached. Clinical specimens were evaluated as positive in melting curve analysis if they showed a maximum –(d/dT) FI in the range of 85 °C to 95 °C that was greater than the maximum –(d/dT) FI of the water negative control.

2.5. Sequencing of 16S rDNA qPCR amplification products

16S rDNA qPCR products that were assessed as positive in fragment or melting curve analysis were purified with the QIAquick PCR purification kit and sequenced using the BigDye kit and an automated DNA sequencer (ABI Prism 3130-Genetic Analyzer, Life Technologies, Zug, Switzerland). Sanger electropherograms were visually examined (regularity of base spacing, distribution of peak heights, occurrence and height of minor background peaks). Any part of the electropherogram that showed irregularities (e.g., high background noise, irregular base spacing, and peak height distributions) was excluded from the reported result. Accurate bacterial identification was achieved by analyzing sequences with a quality score > 20 (">Q20") that covered at least 400 bp of the respective bacterial 16S rRNA gene in the 16S rDNA SmartGene IDNS custom platform (SmartGene, Lausanne, Switzerland). Species- and genus-level identification was done following the criteria published by Bosshard et al. (2003).

Contamination of reagents and materials with traces of bacterial DNA may adversely affect the specificity of broad-range PCR (Loeffler et al., 1999). We used 3 criteria to uncover environmental contamination and to strictly categorize samples as negative: 1) no distinct 16S rDNA PCR fragment could be observed in the CleanGel, and 16S rDNA qPCR fragments revealed lower FI in fragment analysis or lower –(d/dT) FI in melting curve analysis than the water negative control and 2) bacterial identification in a clinical specimen was identical to the identified bacterium in the water negative control (in this study, identification of *Aquabacterium commune* or *Achromobacter* sp. DNA background).

2.6. Statistics

A synopsis of clinical findings (disease history, clinical picture) and laboratory results (microscopy, 16S rDNA PCR, bacterial culture) including consultation with the treating physician was used as gold standard to categorize patients with a "clinically confirmed bacterial infection" or "no bacterial infection." On the basis of this composite diagnostic measure, we used the 2×2 contingency table to calculate the agreement between the 3 different diagnostic workflows (Cohen, 1960; Landis and Koch, 1977).

2.7. Ethics statement

The study was conducted according to good laboratory practice and in accordance with the Declaration of Helsinki and national and institutional standards. The act on medical research involving human subjects does not apply to this study. In this study, solely extracted DNA from clinical specimens and anonymized health-related data were used; therefore, no consent from the ethics committee was required.

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2.8. Availability of data

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

3. Results

3.1. Diagnostic performance of the 16S rDNA qPCR and fragment analysis workflow

In total, 499 clinical specimens were retrospectively analyzed by 16S rDNA qPCR, fragment analysis, and Sanger sequencing and compared to results from the 16S rDNA PCR workflow (Fig. 1). In 362/499 specimens (72%), no bacterial identification was achieved, while in 137/499 specimens (28%), a bacterial pathogen was identified (Table 1). In each assay, 8 specimens from patients with a clinically confirmed bacterial infection were missed (Tables 1, S1).

In 299/499 specimens, no distinct 16S rDNA PCR fragment was visible in the CleanGel, and these specimens were assessed negative. A 16S rDNA PCR amplification product in the CleanGel was present in 200/499 specimens. In 63 of these specimens, bacterial identification was not possible or revealed known environmental contaminants (*Aquabacterium commune* or *Achromobacter* sp.), while bacterial pathogens were identified in 137 specimens. Using fragment analyses, 289/499 specimens revealed an FI that was lower than the FI in the water negative control and therefore were assessed negative. In 210/499 specimens, an FI that was greater than the FI of the water negative control was revealed, and the corresponding PCR products were sequenced (Fig. 2). In 137 of those specimens, a bacterial pathogen was identified, and in 73 of the sequenced specimens, bacterial identification was not possible (i.e., weak peaks or multiple overlying peaks in the Sanger electropherograms) or pointed to known environmental contaminants.

A diverse range of bacterial genera was concordantly identified by 16S rDNA PCR and 16S rDNA qPCR including *Anaerococcus, Actinomyces, Borrelia, Clostridium*, *Enterococcus, Enterobacter, Fusobacterium, Haemophilus*, *Klebsiella, Moraxella, Neisseria, Pseudomonas, Providencia, Streptococcus*, and *Staphylococcus.* The 5 most prevalent bacterial species identified were *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Kingella kingae*.

The 8 bacterial pathogens that were identified by the 16S rDNA PCR but missed by the 16S rDNA qPCR workflow included *Anaerococcus* sp., *Granulicatella adiacens, Cutibacterium acnes, Streptococcus dysgalactiae, Streptococcus intermedius, Yersinia* sp., and 2 specimens with mixed infections (1 specimen with *Porphyromonas endodontalis, Parvimonas micra* and *Clostridium* sp.; 1 specimen with *Enterobacteriaceae* and *Clostridium perfringens*) (Table S1). In 8 specimens, bacterial pathogens were solely identified by the 16S rDNA qPCR workflow and included *Anaerococcus lactolyticus, Enterobacter aerogenes*, *Enterobacter cloacae* complex, *Kingella kingae, Cutibacterium acnes, Staphylococcus epidermidis, Streptococcus mitis/oralis* group, and *Streptococcus pneumoniae* (Table S1). In 2 of these specimens, culture confirmed the results obtained by the 16S rDNA qPCR workflow. In 3 specimens, culture was found negative, and in the remaining specimens, culture was not performed.

Table 1
Comparison of the performance of the old (16S rDNA PCR, CleanGel analysis, ABI sequencing) and the new diagnostic workflow (16S rDNA qPCR, fragment analysis, ABI sequencing) in 499 clinical specimens. A composite diagnostic measure combining clinic and microbiology results was used as gold standard.

		Clinical and microbiological data	
		Positive	Negative
16S rDNA PCR, CleanGel, sequencing	Positive	137	0
	Negative	8	354
16S rDNA qPCR, fragment analysis, sequencing	Positive	137	0
	Negative	8	354

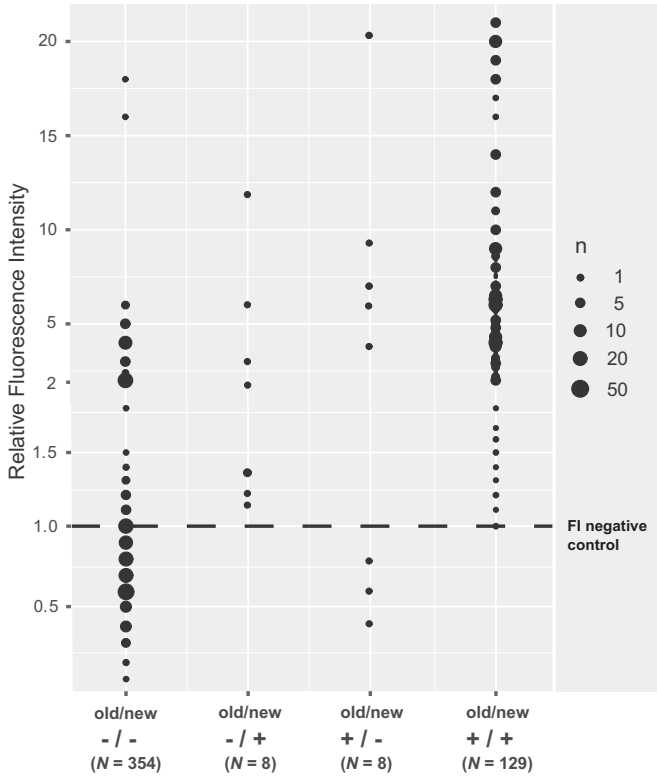

Fig. 2. Relative fluorescence intensities (FI of the sample/FI of the water negative control) of the 16S rDNA qPCR amplification products in fragment analysis are displayed and categorized based on the results from the old (16S rDNA PCR, CleanGel, sequencing) and the new (16S rDNA qPCR, fragment analysis, sequencing) diagnostic workflows.

Overall, a similar proportion of negative specimens were unnecessarily sequenced in the 16S rDNA PCR and 16SrDNA qPCR and fragment analysis workflow, and also sensitivity was comparable as both workflows did not identify bacterial pathogens in an equal number of clinical specimens. The agreement of the old 16S rDNA PCR and the new 16S rDNA qPCR and fragment analysis workflow for the diagnosis of bacterial infections was 97% using a composite diagnostic measure combining clinic and microbiology results as the gold standard (Table 1).

3.2. Diagnostic performance of the 16S rDNA qPCR and melting curve analysis workflow

A subset of 330 of the 499 clinical specimens was analyzed by applying all 3 diagnostic workflows (Fig. 1, Table 2).

In melting curve analysis, 146/330 specimens showed a maximum –(d/dT) FI that was lower than the –(d/dT) FI of the water negative control and therefore were assessed negative. In 184/330 specimens, a –(d/dT) FI that was greater than the –(d/dT) FI of the water negative control was shown, and the corresponding 16S rDNA qPCR products were

Table 2
Comparison of the performance of 3 diagnostic workflows for bacterial pathogen detection in 330 clinical specimens. A composite diagnostic measure combining clinic and microbiology results was used as gold standard.

		Clinical and microbiological data	
		Positive	Negative
16S rDNA, Clean Gel, sequencing	Positive	108	0
	Negative	5	217
16S rDNA qPCR, fragment analysis, sequencing	Positive	111	0
	Negative	2	217
16S rDNA qPCR, melting curve analysis, sequencing	Positive	103	0
	Negative	10	217

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subsequently sequenced (Fig. 3). In 103 of those specimens, a bacterial pathogen was identified, and in 81 of the sequenced specimens, bacterial identification was not possible (i.e., weak peaks or multiple overlying peaks in the Sanger electropherograms) or pointed to known environmental contaminants (*Aquabacterium commune* or *Achromobacter* sp.; $N = 12$).

In 10 specimens, bacterial pathogens could not be identified by the 16S rDNA qPCR and melting curve analysis workflow and included *Anaerococcus lactolyticus, Anaerococcus octavius* ($N = 2$), *Borrelia afzelii*, *Granulicatella adiacens*, *Kingella kingae, Staphylococcus aureus* ($N = 2$), *Streptococcus gordonii*, and *Streptococcus intermedius* (Table S2).

Overall, melting curve analysis showed unspecific detection of 16S rDNA qPCR fragments, and consequently a high proportion (25%) of negative specimens were unnecessarily sequenced. Moreover, melting curve analysis missed 10 specimens from patients with clinically confirmed bacterial infections and therefore showed less sensitive detection of 16S rDNA qPCR fragments than CleanGel and fragment analysis.

4. Discussion

Rapid detection of bacterial pathogens results in improved patient management, earlier initiation of effective treatment, a decrease in hospitalization time, and reduced costs for the health care system (Maurer et al., 2017). The primary aim of this study was to assess a more rapid 16S rDNA qPCR and fragment analysis screening for the diagnosis of acute bacterial infections and to provide evidence-based data showing that gel-based visualization methods can be replaced. The proposed diagnostic workflow offers a higher degree of automation and standardization and improves routine diagnostics by lowering turnaround time. Furthermore, it enhances quality and reproducibility by reducing interobserver variability. Automated DNA extraction (batches of 24 clinical samples can be analyzed per run) and pipetting of the qPCR setup (i.e., on the QIASymphony) reduce confusion of samples and errors occurring while pipetting the samples (reagents are not added, too little/high volumes are added, etc.). Reports are created automatically on the fragment analyzer, and assessment of sample positivity as shown in Fig. 2 can be performed with simple data analysis tools like Excel. In contrast, the CleanGel needs visual inspection and evaluation if 16S rDNA PCR bands from clinical specimens are stronger than the negative control band, which leaves great scope for personal interpretation and leads to high interobserver variability.

When comparing all 3 diagnostic workflows, 16S rDNA qPCR in combination with fragment or melting curve analysis offers the most rapid pathogen detection (less than 1 working day to assess a clinical specimen as negative and 1.5 days to identify the bacterial pathogen by 16S rDNA sequencing). In contrast, the 16S rDNA PCR workflow takes 2 days to assess specimens as negative and 2.5 days until bacterial identification is completed. When comparing the overall costs of the different workflows, the 16S rDNA PCR workflow had the highest per-sample cost (20 € for DNA extraction chemicals and PCR reagents [including controls] and 16 € for Sanger sequencing), followed by the 16S rDNA qPCR and fragment analysis workflow (15 € for extraction chemicals and PCR reagents [including controls] and 16 € for Sanger sequencing) and the 16S rDNA qPCR and melting curve analysis workflow (13 € for extraction chemicals and PCR reagents [including controls] and 16 € for Sanger sequencing).

In all 3 workflows, there were a high proportion (13% to 25%) of sequenced specimens that yielded uninterpretable sequences or were identified as environmental contaminants. This emphasizes the need for proper controls in the 16S rDNA PCR and 16S rDNA qPCR, respectively, in order to avoid false-positive identifications due to background DNA from chemicals and reagents. Moreover, further attempts should be undertaken to reduce the DNA background in chemicals and reagents used for broad range applications.

The 16S rDNA qPCR and melting curve analysis workflow revealed the lowest sensitivity, whereas the other 2 workflows revealed identical

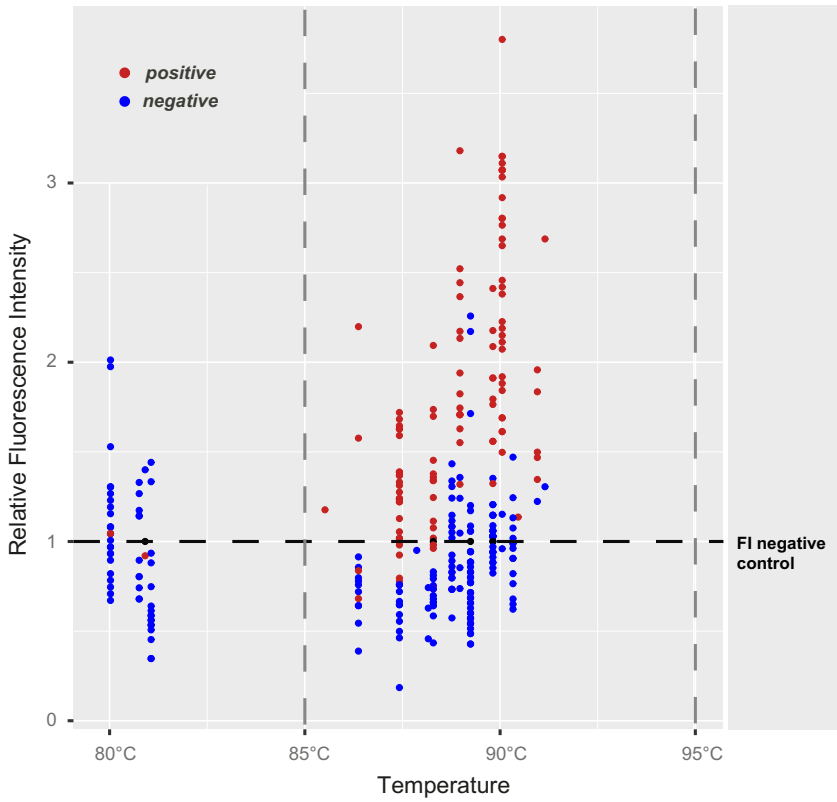

Fig. 3. Relative fluorescence intensities (maximum −(d/dT) FI of the sample/ maximum −(d/dT) FI of the water negative control) of the 16S rDNA qPCR amplification products in melting curve analysis are displayed and categorized as positive or negative based on a composite diagnostic measure combining clinic and microbiology results.

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sensitivity. Eight clinical specimens were assessed as positive in the 16S rDNA PCR and negative in the 16S rDNA qPCR and fragment analysis workflow. Three of these 8 specimens showed a fluorescence intensity in fragment analysis that was lower than the fluorescence intensity of the water negative control. Review of the original silver stained CleanGel showed rather weak PCR bands for these three samples. If the DNA yield is low in the clinical specimen, prolonged freezing and thawing of genomic DNA may lead to progressive DNA degradation (Shao et al., 2012) and potentially explain the inability to identify the bacterial pathogens by 16S rDNA qPCR and subsequent sequencing.

One advantage of agarose or polyacrylamide-gel-based detection methods is that single bands can be cut from the gel, thereby enabling species identification even in mixed infections. Newer bioinformatic tools like the RipSeq web application (Pathogenomix, Santa Cruz, CA, USA) can be used to analyze overlaying DNA chromatograms but in our experience only delivered bacterial species identification with a sufficient degree of certainty if mixed samples did not contain more than 2 different species. In our opinion, this disadvantage of the 16S rDNA qPCR and fragment analysis workflow is negligible as mixed infections occurred only in 2 of 499 clinical specimens.

5. Conclusion

The 16S rDNA qPCR and fragment analysis workflow has a fast time-to-result (less than 1 working day to assess a clinical specimen as negative and less than 1.5 days to obtain species identification by 16S rDNA sequencing). A key advantage of this new workflow is that it does not require time-consuming seminested PCR and CleanGel analysis. Moreover, it allows for a high degree of automation and standardization as sample positivity is assessed using bioinformatic procedures in fragment analysis.

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Declaration of interest

All authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diagmicrobio.2018.11.006.

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